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CELL CULTURING DEVICE
[Saibo baiyo sochi]

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UNITED STATES PATENT AND TRADEMARK OFFICE
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Specifications /533*

1. Title of the Invention

Cell Culturing Device

2. Claim

(1) A cell culturing device equipped with a gas control system wherein gas is supplied over to contact the surface of a culture solution housed in a culture tank containing cells and a dissolved oxygen concentration sensor is submerged in the culture solution to control the supply of the gas over the surface of the culture solution with the aid of a control part by means of a detection signal from the dissolved oxygen concentration sensor, a level control system which actuates a medium-exchanging pump through the control part according to a signal from a level sensor when some of the culture solution is removed and feeds a fresh medium to the culture tank, an optical density sensor which illuminates the culture solution with light to measure the cell density from the absorbance thereof, and a level sensor having an actuator for changing the set value of the liquid level due to an instruction from the control part; the aforesaid control part is equipped with, in addition to each of the aforesaid functional parts, a growth rate-calculating part which calculates the growth rate as an amount of change in output from the aforesaid density sensor, a culture solution quantity decision part which

*Number in the margin indicates pagination in the foreign text.

determines the amount of increase in the culture solution from the output of the aforesaid density sensor and the growth rate to control driving of the aforesaid actuator, a medium replacement quantity decision part which determines the amount of medium to be replaced according to the output of the aforesaid density sensor as well as the growth rate to control driving of the aforesaid medium-exchanging pump, and a dissolved oxygen concentration decision part which increases the concentration of the dissolved oxygen via the gas control system if the output from the aforesaid density sensor is at or greater than a prescribed value.

3. Detailed Specifications

(Field of Industrial Utilization)

The present invention pertains to a device for culturing cells, and in particular, a cell culturing device which maintains a suitable dissolved oxygen concentration (DO) and automatically exchanges the culture solution to supply nutrients and remove wastes.

This kind of cell culturing device is utilized as a high-density cell culturing device which cultures highly dense cells, for example.

(Prior Art)

In a high-density cell culturing device, a gas is supplied over to contact the surface of a culture solution housed in a

culture tank containing cells. A pH sensor, dissolved oxygen concentration sensor, and temperature sensor were submerged in the culture solution, a level sensor which detects the level of /534 the liquid is provided, the amount of CO_2 (gas) supplied over the surface of the culture solution is controlled according to the detection signal from the pH sensor, the amount of O_2 (gas) supplied over the surface of the culture solution is controlled according to the detection signal from the dissolved oxygen concentration sensor, power is supplied to a heater for heating the culture solution according to the detection signal form the temperature sensor, a fresh medium pump is actuated according to the detection signal from a level sensor when some of the culture solution is removed, fresh medium is fed to the culture tank, and the amount of culture solution in the culture tank is kept constant.

The cell density increases as culturing proceeds. As the cell density increases, a person sets the environmental conditions by removing some of the culture solution to outside the system and then changes the environmental conditions after measuring the cell density.

(Problems Which the Invention Intends to Solve)

There is a close relationship between the growth rate and environmental conditions when cells are cultured. If the environmental condition settings are made in error, there is an

adverse effect on the growth of the cells after that.

The factors for the environmental conditions include temperature, pH, dissolved oxygen concentration, amount of culture to be exchanged, rotating speed of the stirrer, amount of culture solution, etc. Optimizing these conditions is difficult work for a person while he/she is measuring the cell density.

The object of the present invention is to automatically perform changes in such environmental conditions.

(Means Used to Solve the Problems)

The present invention will be explained by referring to Figs. 1 and 3.

The cell culture device of the present invention gas is equipped with a gas control system wherein gas is supplied over to contact the surface of a culture solution **4** accommodated in a culture tank **2** and a dissolved oxygen concentration sensor **12** is submerged in the culture solution **4** to control the supply of the gas over the surface of the culture solution **4** with the aid of a control part **42** according to a detection signal from the dissolved oxygen concentration sensor **12**, a level control system which actuates medium-exchanging pumps **80** (**50**, **54**) with the aid of the control part **42** according to a signal from a level sensor **14** when some of the culture solution **4** is removed and feeds a fresh medium to the culture tank **2**, an optical density sensor **70** which illuminates the culture solution **2** with light to measure

the cell density from the absorbance thereof, and a level sensor **14** having an actuator **76** for changing the set value of the liquid level due to an instruction from the control part **42**. The control part **42** is equipped with, in addition to each of the aforesaid functional parts, a growth rate-calculating part **72** which calculates the growth rate as an amount of change in output from the density sensor **70**, a culture solution quantity decision part **74** which determines the amount of increase in the culture solution from the output of the density sensor **70** and the growth rate to control driving of the actuator **76**, a medium replacement quantity decision part **78** which determines the amount of medium to be replaced according to the output of the density sensor **70** as well as the growth rate to control driving of the medium-exchanging pump **80** (**50**, **54**), and a dissolved oxygen concentration decision part **82** which increases the concentration of the dissolved oxygen via a gas control system **84** if the output from the density sensor **70** is at or greater than a prescribed value.

(Operation of the Invention)

Figure 2 shows the results of a continuous high-density culturing experiment using a serum-free medium. The Y axis is the live cell density and the X axis is the culturing time.

The cells that were used were NAT-30 (human lymphoid cells). The basal medium is (RPMI-1640)+DME+(F-12). The growth factors

and the like include insulin, transferrin, ethanolamine, serine, lipoproteins, and serum albumin. The set conditions include a pH of 7.2, a temperature of 37°C, stirring speed of 60 r.p.m., a dissolved oxygen concentration of 5.0 ppm in periods **ta** and **tb**, and a concentration higher than that in period **tc**. If the maximum culture solution volume in the culture tank was 1.0 L, the culture solution replacement rate was 0 in period **ta** and 2.0 L/day in periods **tb** and **tc**.

The results shown by the solid curve in Fig. 2 were controlled manually by the discernment of the tester, but this is a situation in which the setup of the environmental conditions was performed skillfully.

If replacement of the medium is performed when it is at its lowest density (in period **ta**) after inoculating the medium with the cells, the cells die or decrease in number, as shown by the dashed curve **A**.

In addition, if exchange of the culture solution is performed in a logarithmic growth phase (until there is no further increase in the live cell density in periods **tb** and **tc**), the cells still continue to die, as shown by the dashed curve **B**.

And, if the dissolved oxygen concentration increases in period **tc** when the live cell density is the highest, the live cell density reaches a state where it does not increase adequate, as shown by curve **C**.

Then, in the present invention, the cell density is automatically measured by the density sensor **70** and control is automatically performed so that the increase in the cell density /535 is edified according to the cell density and growth rate, as shown by the solid curve in Fig. 2.

For that purpose, based on the experimental results, as shown in Fig. 2, data is given to the control part **42**. The amount of the culture solution, the amount of medium to be replaced, and the dissolved oxygen concentration are automatically set according to the cell density and growth rate so as to get the resulting cell density, as shown by the solid curve.

An increase in the amount of culture solution is realized by driving an actuator **76** of the level sensor **14** is driven according to a signal from a culture solution increment decision part **74**. If the level sensor **14** rises above the existing level of the culture solution **4**, the amount of the culture solution **4** is increased as far as that raised position by a medium-exchanging pump **80**.

When the amount of medium replaced changes, the medium-exchanging pump **80** is driven by a signal from a medium exchange quantity decision part **78**.

When the dissolved oxygen concentration increases, a gas control system **84** is controlled by a signal from a dissolved

oxygen concentration decision part **82**, and the oxygen pressure in the atmosphere coming in contact with the surface of the culture solution **4** is elevated.

(Practical Examples)

Figure 3 shows a practical example.

2 is a culture tank where the culture solution **4** is accommodated. Cells are contained in the culture solution **4**. The upper part of the culture tank **2** is closed airtight by a cover **6**, and the culture solution **4** contacts the gas in the space above the culture solution **4**.

The temperature sensor **8**, pH sensor **10** and dissolved oxygen concentration sensor **12** are submerged in the culture solution **4** via the cover **6**. A gas supply pipe **16** for supplying gas and a vent pipe **18** for evacuating the gas are provided in the cover **6** and in the space above the culture solution **4**.

The level sensor **14** is provided outside the culture tank **2** in order to detect the level of the culture solution **4**.

O_2, CO_2 and N_2 (or air) are supplied through the gas supply pipe **16** via respective regulators **22-1** to **22-3**, solenoid valves **24-1** to **24-3** and check valves **26-1** to **26-3**. **28** is a filter and **40** is a safety valve. An exhaust nozzle **30**, check valve **32**, orifice **34** and solenoid valve **36** are connected to the vent pipe **18**. **38** is a gauge provided in an exhaust pipe.

Opening and closing of the solenoid valve **24-1** for supplying O_2 is controlled by the control part **42** according to a detection signal from the dissolved oxygen concentration sensor **12** so that the dissolved oxygen concentration becomes a set value. Opening and closing of the solenoid valve **24-2** for suppling CO_2 is controlled by the control part **42** according to a detection signal from the pH sensor **10** so that the pH becomes a set value. The solenoid valve **24-3** for supplying N_2 is used to decrease the O_2 and CO_2 partial pressures inside the culture tank **2** and to displace the O_2 and CO_2 gas in the culture tank **2**. The opening and closing are controlled by the control part **42** in accordance with the detection signals from the pH sensor **10** and the dissolved oxygen concentration sensor **12**.

The gas control system is constituted from the dissolved oxygen concentration sensor **12** and the solenoid valves **24-1** and **24-3** and the pH control system is constituted from the pH sensor **10**, and the solenoid valves **24-2** and **24-3**.

A heater **44** is provided in the lower part of the culture tank **2**. Supplying power to the heater **44** is controlled by the control part **42** according to a detection signal from the temperature sensor **8** so that the temperature becomes constant.

The culture solution **4** is circulated between the culture tank **2** and a cross-flow filter **48** by a perfusion pump **46**. The

cross-flow filter **48** is a cylindrical filter made from a ceramic. The culture solution containing the cells passes through the filter **48**. Some of the culture solution is filtered outside the filter **48** and drained. The filtered culture solution is removed to outside the culturing device system by a spent medium pump **50**. **52** is the removed spent medium. A fresh medium **56** is fed from outside the system over the culture solution **4** by a fresh medium pump **54**. The perfusion pump **46**, spent medium pump **50** and fresh medium pump **54** are controlled by the control part **42**.

The level sensor **14** detects the level of the culture solution **4**. If the culture solution is drained via the cross-flow filter **48**, the level of the culture solution **4** inside the culture tank goes down; therefore, the control part **42** does control by actuating the fresh medium pump **54** according to the detection signal of the level sensor **14** so that the fresh medium /536 **56** is fed to the culture tank **2** and the level of the culture solution **4** attains the position of the level sensor **14**.

The level sensor **14**, control part **42**, perfusion pump **46**, spent medium pump **50** and fresh medium pump **54** constitute a level control system.

A stirrer **58** is provided in the culture solution **4** and a magnet **62** is provided in the lower part of the culture tank **2**. The stirrer **58** is actuated by turning the magnet **62** with the aid

of a stirrer motor **60**. The stirrer motor **60** also is controlled by the control part **42**.

64 is the display part of the control part **42** for displaying the temperature, pH, dissolved oxygen concentration, etc. **66** is a setting part for setting the set values of the temperature and the like in the control part **42**.

The optical density sensor **70** shown in Fig. 4 is provided in the flow passage containing the perfusion pump **46** and cross-flow filter **48**.

A transparent glass cell **89** is provided between silicon rubber tubes **86** and **87** constituting the flow passage of the density sensor in Fig. 4, and the culture solution passes through the cell **89**. Light is illuminated from an LED **90** on one side and the light transmitting through the cell **89** is received by a photodiode **91** on the other side of the cell **89**. A sensor used for finding the density of the suspension from the amount of photoabsorption by the suspended sample using light is generally used as a turbidity sensor. Using the same principles as that turbidity sensor is the density sensor in this practical example.

Figure 5 shows the relationship between the cell density and output from the density sensor **70** in a 1-to-1 relationship.

The level sensor **14** can automatically change the set value of the liquid level; one example thereof is shown in Fig. 6.

92 is an LED and 93 is a photodiode which are supported by a support member 94 so that they face each other with the culture tank 2 between them. The support member 94 is supported by a guide 95 to be vertically movable. In addition, a screw 96 is provided in the support member 94. A rod screw 97 turned by a pulse motor 98 and the screw 96 thereof are screwed together.

The support member 94, screws 96 and 97, and pulse motor 98 become the actuator of the level sensor 14. When the support member 94 is moved vertically by actuation of the pulse motor 98, the position that it is moved to becomes the new liquid level set value and the level of the culture solution 4 is changed by the medium-exchanging pumps 50 and 54.

The fact that the intensity of the light illuminated from the LED 92 and incidented on the photodiode 93 changes due to the position of the level of the culture solution 4 is utilized by this liquid level sensor 14. If the liquid surface is lower than the optical path of the light illuminated from the LED 92 and incidented on the photodiode 93, the light intensity of the received light decreases and the liquid surface rises. When the optical path passes through the liquid, the optical path bends by refraction and the light intensity decreases.

Figure 7 shows the connecting relationship between the control part 42 and the various sensors and drive portions.

The control part **42** is constituted from a CPU **42a** and a knowledge data base **42b** utilized for making set values from the experimental results for setting the environmental conditions, as shown in Fig. 2. Data can be further added to the environmental conditions that are already set in the knowledge data base **42b**.

The operation in a practical example will be explained next through Fig. 8.

The cells are inoculated (Step S1), and the dissolved oxygen concentration, pH, temperature and number of stirrers are set so that they are set values. The density **D** is measured (Step S2) and the growth rate **R** is calculated form the rate of change in the density **D** (Step S3).

The density **D** is compared with the 1st density $\mathbf{D_1}$ (e.g., 1×10^6 cells/mL) set according to the knowledge data base (Step S4). When the density **D** is less than $\mathbf{D_1}$, the medium is not replaced (Step S5). And, no increase in the amount of culture solution is made either (Step S6).

If culturing continues, before long the density $\mathbf{D>D_1}$ (Step S4). When the density **D** is less than a 2nd density $\mathbf{D_2}$ (1×10^7 cells/mL), the dissolved oxygen concentration is kept constant as is (Step S8). If the growth rate **R** exceeds 50%, the amount of medium replaced is 500 mL per day (Step S10, S11), and the amount of medium increased is 1/5 of the total amount of the culture solution per day (Step S12) provided that the maximum volume of /537

the culture solution able to be accommodated in the culture tank is 500 mL.

When the density **D** exceeds the 2nd density set value $\mathbf{D_2}$ as the culturing continues further, the dissolved oxygen concentration increases after that time (Steps S7 and S9). In increasing the dissolved oxygen concentration, the pressure in the upper part of the culture solution inside the culture tank increased by 0.5 kg/cm^2. When the growth rate **R** exceeds 50%, the amount of culture solution replaced is 500 mL/day, the amount of increase is 1/5 of the total amount of culture solution per day (Steps S10, S11 and S12), and culturing proceeds.

If the cell density approaches a saturated state before long, the growth rate is less than 50%. Subsequently, the amount of culture solution does not increase and the amount of medium replaced is 1,000 mL/day (Steps S10, S13 and S6). The culturing carries on at this condition hereafter.

(Advantages of the Invention)

The density of the culture solution was automatically measured by the density sensor in the present invention, and it was controlled so that the amount of the medium replaced, amount of the culture solution increased, and the dissolved oxygen concentration automatically change according to the measured density as well as the growth rate, which is the amount of change in density; therefore, the culturing process started from a small

amount of culture solution and number of cells can be automated to perform a mass culture.

4. Brief Description of the Figures

Figure 1 is a block diagram showing the portions related to the control part of the present invention; Figure 2 is a block diagram showing the results of a culturing experiment; Figure 3 is a block diagram showing a practical example; Figure 4 is a schematic perspective view showing the density sensor; Figure 5 is a drawing showing the relationship between the cell density and density sensor output; Figure 6 is a schematic perspective view showing an example of a level sensor; Figure 7 is a block diagram showing the connecting relationship between the control part and the drive part of a practical example; Figure 8 is a flowchart showing the operation in a practical example.

2... culture tank; 4... culture solution; 12... dissolved oxygen concentration sensor; 14... level sensor; 42... control part; 70... density sensor; 72... growth rate calculation part; 74... culture solution increment decision part; 76... level sensor actuator; 78... medium exchange quantity decision part; 80... medium-exchanging pump; 84... gas exchange system.

[Figure 1] /538
(70) optical density sensor;
(82) dissolved oxygen concentration decision part;
(84) gas control system;
(74) culture solution increment decision part;
(76) level sensor actuator;
(72) growth rate calculation part;
(78) medium exchange quantity decision part;
(80 (50, 54)): medium-exchanging pumps

[Figure 2]
Key: (a) Live cell density; (b) culturing time; (c) start of pressure increase; (d) Start of medium replacement

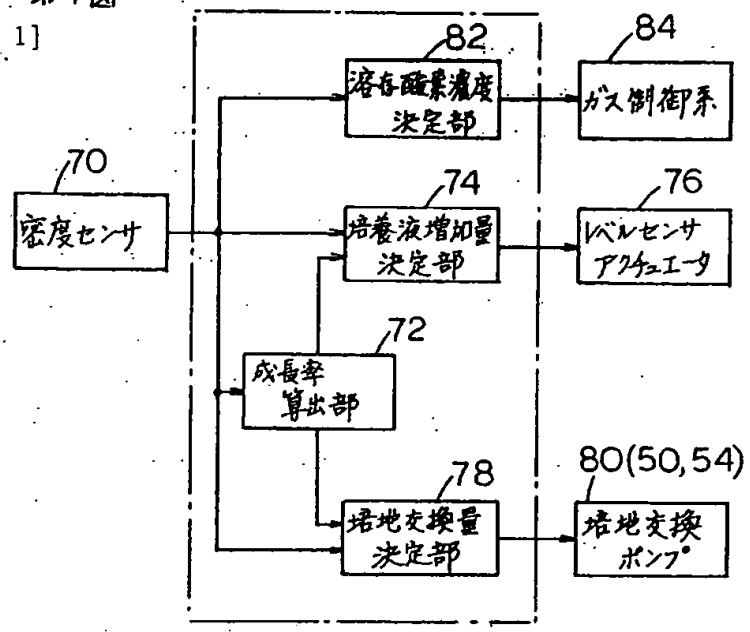
[Figure 3] /539
(64) display part;
(66) setup part;
(42) control part;
Key: (a) exhaust.

[Figure 5] /540
Key: (a) cell density (cells/mL); (b) sensor output

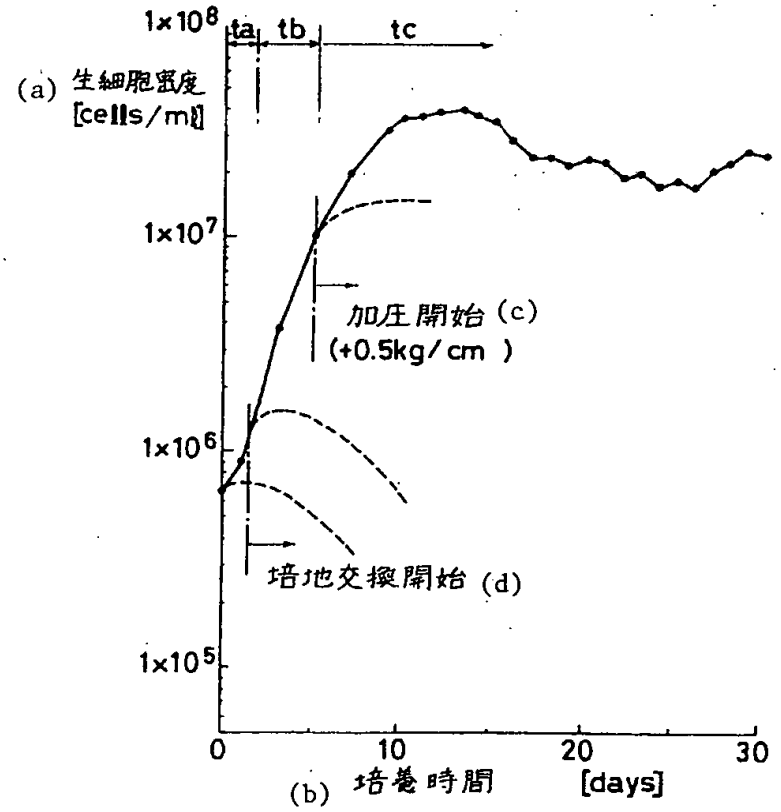
[Figure 7] /541
(70) density sensor;
(14) level sensor;
(10) pH sensor;
(12) DO sensor;
(8) temperature sensor;
(42b) knowledge data base;
(98) level sensor actuator;
(46) perfusion pump;
(80) medium-exchanging pump;
(24-1 to 24-3) gas controls.

[Figure 8]
(S1) Inoculate with cells;
(S2) Measure density **D**;
(S3) Calculate growth rate **R**;
(S9) Increase DO;
(S8) DO constant?;
(S11) Replacement quantity: 500 mL/day;
(S13) Replacement quantity: 1,000 mL/day;
(S5) Replacement quantity: 0;
(S12) Amount of increase: 1/5/day;
(S6) Amount of increase: 0;
Key: (a) Start; (b) no; (c) yes.

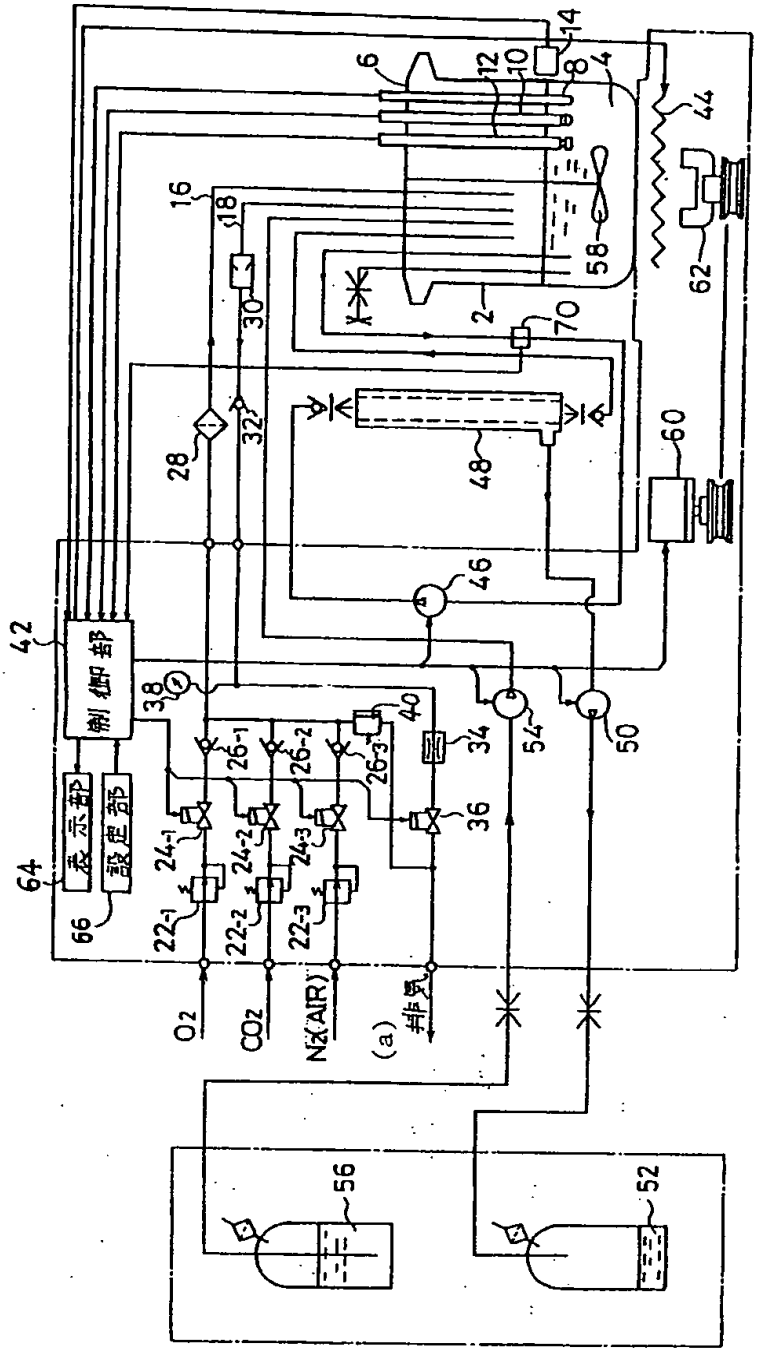
第1図
[Figure 1]



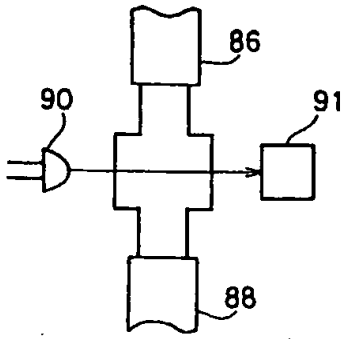
第 2 図 [Figure 2]



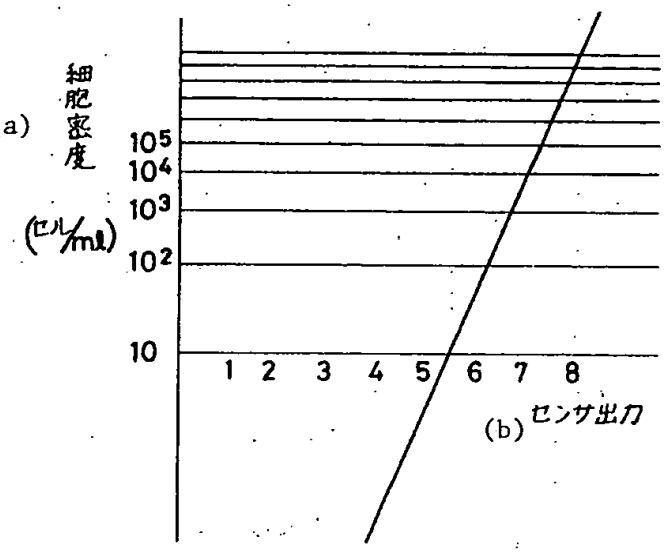
第3図 [Figure 3]



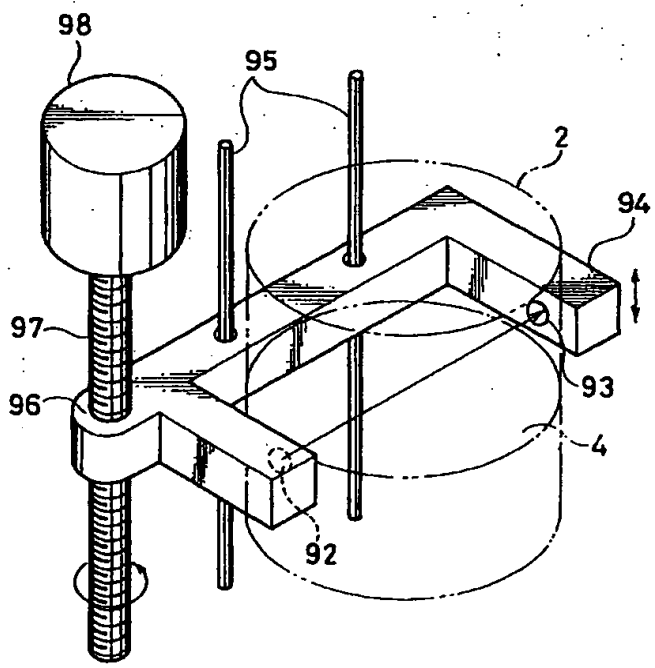
[Figure 4]　第 4 図



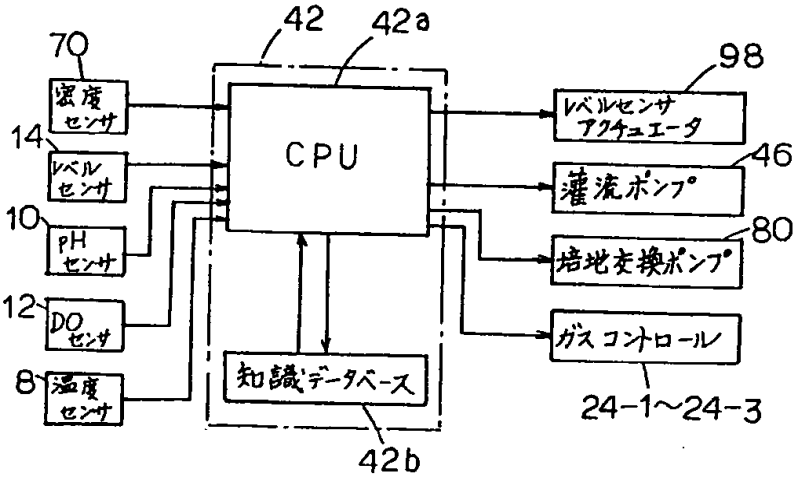
[Figure 5]　第 5 図



第 6 図 [Figure 6]



第7図 [Figure 7]



[Figure 8] 第8図